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## PHOTOPERIODIC INDUCTION OF DIAPAUSE IN AN INSECT<sup>1</sup>

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Diapause is defined as a state of arrested development in which the arrest is enforced by a physiological mechanism rather than by concurrently unfavorable environmental conditions. Although not maintained directly by environmental factors, diapause is apparently induced, and in many species also terminated, in response to environmental stimuli. Most insect species are capable of diapause at some stage in the life cycle; embryonic, larval, pupal, and adult diapauses have been reported. Since the classical study of Kogure (1933) on the role of photoperiod in the induction of embryonic diapause in the silkworm, *Bombyx mori* L., daylength has been reported as a major inducing factor in the diapause of many other species. The recent profusion of reviews and symposia on the subjects of diapause and photoperiodism makes a detailed review of literature undesirable here; see Lees (1955, 1959, 1960), Bunning (1960), and Harker (1960b, 1961).

The present study was undertaken to demonstrate some of the characteristics of photoperiods effectively inducing diapause in the European corn borer, *Ostrinia nubilalis* (Hbn.). Larval diapause in this species has been shown to be a response to short days and low temperatures (Mutchmor and Beckel, 1959; Beck and Hamec, 1960). These earlier studies involved measurement of the effects of ecologically possible photoperiods, *i.e.*, a 24-hour total cycle. Under such limited experimental conditions, it was not possible to determine the relative importance of the light and dark phases of the over-all photoperiod, because one phase could not be varied except at the expense of the other. Neither was it possible to determine whether or not the photoperiodic response involved an endogenous circadian rhythm within the organism, as postulated by Bunning (1960) or a non-circadian "interval timer," as postulated by Lees (1960a, 1960b). The experimental work reported below was designed to contribute to the clarification of these problems.

### MATERIALS AND METHODS

The European corn borers used in this study were from a restricted natural population occurring near Madison, Wisconsin. The use of a defined population

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was necessary because of the demonstration of significant differences in photoperiodic responses among different geographical populations of this species (Beck and Apple, 1961). Overwintering borers were collected from the field in the fall of the year, and were stored at 4° C. As needed, groups of the stored borers were placed at 30° C. for pupation and emergence. The progeny of these insects were used in the experiments described below.

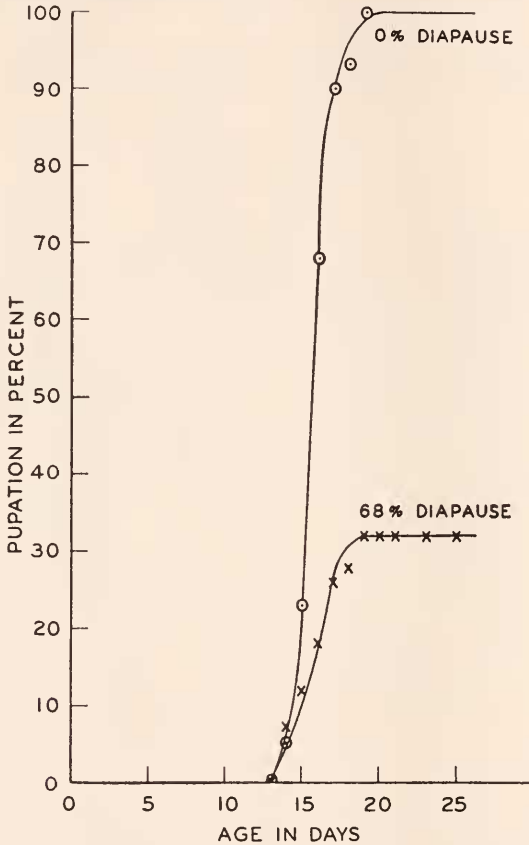


FIGURE 1. Typical pupation records of experimental populations of the European corn borer, illustrating the method of determining incidence of diapause.

The experimental borers were reared aseptically on purified diets, according to the rearing techniques described by Beck and Smissman (1960). Photoperiod experiments were run at 30° C., and the larvae were exposed to the experimental photoperiods continuously from an age of less than 24 hours. Each experiment involved the use of 250 newly hatched larvae. The larvae were divided into 5 groups of 50 each; one group was reared in continuous dark as the experimental control; the other 4 groups were exposed to the experimental conditions and were treated statistically as replicates. Because of mortality and microbial contamination, the final number of insects in each replicate and the control varied from 40

to 46. As the larvae reached maturity, daily pupation records were taken. Diapause incidence was measured as the per cent of mature larvae that failed to pupate. This was determined on the basis of the sigmoid configuration of the pupation record (Fig. 1); when the curve had remained unchanged for several days, pupation was considered to have been completed. The remaining mature larvae were considered to be in diapause.

The experiments were carried out in B.O.D. constant temperature incubators that had been modified to incorporate a thermistor temperature control system (Thermistemp Temperature Controller Model 71, Yellow Springs Instrument Company, Yellow Springs, Ohio). Control of photoperiod was effected through the use of 7-day cycle programmers wired to two 14-watt fluorescent lights in-

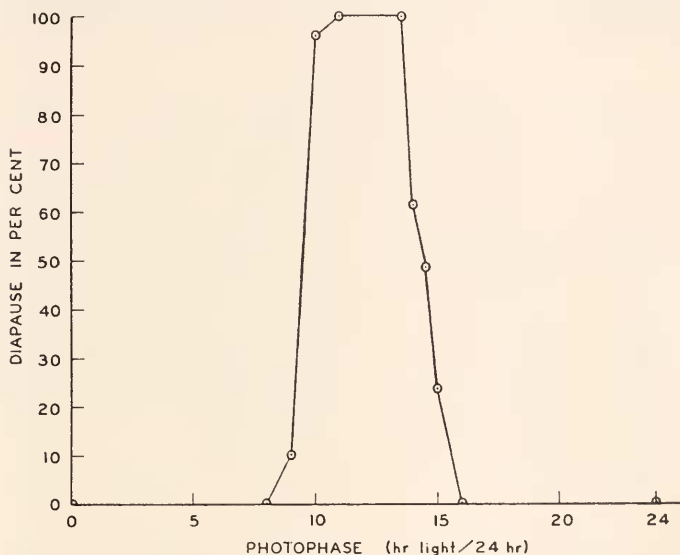


FIGURE 2. Effect of photophase duration on diapause incidence among European corn borer larvae reared under 24-hour photoperiods.

stalled in the incubator. In experiments involving temperature changes, a 24-hour temperature cycle was effected by using a clock motor to drive the thermistor temperature control unit through a prescribed cycle. The performance of the cycling apparatus was verified by the use of a recording thermograph.

In the discussion of the results, which follows, the term *photoperiod* is restricted to refer to the total cycle composed of a period of light and a period of dark. The period of light within the photoperiod is referred to as the *photophase*; conversely, the dark portion of the photoperiod is termed the *scotophase*. This terminology is recommended as a means of avoiding the usual ambiguous use of the term photoperiod. It has been commonly used to refer to (1) the total light/dark cycle, and (2) only the light portion of the total cycle. Since the structure of the word implies a periodicity involving light, and since the only periodic alternative to light is dark, the term should be used only in the sense of the total light/dark cycle.

## RESULTS

At an incubation temperature of 30° C. and a photoperiod of 24 hours, the effect of photophase duration on the incidence of diapause in the European corn borer is shown in Figure 2. The effective range of photophases was between 8 and 16 hours, with diapause incidence exceeding 90% only under photophases of from 10 to 14 hours. The borer is a "long-day" insect, in the classical sense of the term (Dickson, 1949; Lees, 1952; Otuka and Santa, 1955). It should be noted, however, that the term "long-day insect," in the sense of an insect that develops without interruption under the influence of 24-hour photoperiods containing a relatively long photophase (> 15 hr.), is a misnomer. Abnormally short photophases (< 9 hr.) also result in uninterrupted development in this and some other species. The diapause incidences obtained (Fig. 2) were in agreement with those reported

TABLE I  
*Effect of photophase:scotophase ratios on incidence of diapause among European corn borer larvae*

Photophase:Scotophase = 1:1				Photophase:Scotophase = 2:1				Photophase:Scotophase = 1:2			
Photo. (hr.)	Scoto. (hr.)	Total (hr.)	Diapause (%)	Photo. (hr.)	Scoto. (hr.)	Total (hr.)	Diapause (%)	Photo. (hr.)	Scoto. (hr.)	Total (hr.)	Diapause (%)
6	6	12	<5	10	5	15	<5	4	8	12	<5
8	8	16	<5	12	6	18	<5	5	10	15	32
9	9	18	78	14	7	21	<5	6	12	18	95
10	10	20	92	16	8	24	<5	7	14	21	65
12	12	24	100	20	10	30	31	8	16	24	<5
14	14	28	85	24	12	36	65				
16	16	32	50	28	14	42	<5				
18	18	36	19								
20	20	40	<5								

in earlier studies of diapause in the European corn borer (Mutchmor and Beckel, 1959; Beck and Hanec, 1960), and are of interest to the present study only in that they delimit the responses of the insect to naturally occurring photoperiods.

Diapause incidence under 24-hour photoperiods approached a maximum when the photophase:scotophase ratio was approximately 1:1, and approached a minimum at ratios of either 1:2 or 2:1. Diapause incidence under photoperiods displaying different photophase:scotophase ratios is shown in Table I. One-to-one ratios were ineffective when the phase durations were either under 8 or over 16 hours. Diapause incidence was very high when both photophase and scotophase were from 9 to 14 hours, which is similar to the responses obtained in the series of 24-hour photoperiods (Fig. 2). When the duration of the photophase was twice that of the scotophase (2:1 series, Table I), diapause was induced only when the scotophase was from 10 to 12 hours. When the scotophase was twice as long as the photophase (1:2 series, Table I), diapause was evoked only by scotophases of from 10 to 14 hours. Total photoperiod duration did not appear to influence the incidence of diapause in these experiments; nor did the phase ratios appear to be of any significance. Diapause induction was closely associated with scoto-

phases of from 10 to 14 hours, with maximum effectiveness at 12 hours. These findings are in agreement with those of Danilyevsky and Glinyanaya (1950), who worked with *Acronycta* spp. Otuka and Santa (1955), experimenting with *Barathra brassicae* L., concluded that, although the absolute length of the phases was of importance, phase ratio also influenced diapause induction; their data on this point are not all convincing, however, as they tested only 1:1 ratios.

A series of experiments was run in which photophases of 10, 12, and 14 hours were combined with a wide range of scotophases (Fig. 3). At these photophases, the range of diapause-inducing scotophases was relatively narrow; scotophases of from 10 to 14 hours were required to produce a diapause incidence of 90 or more

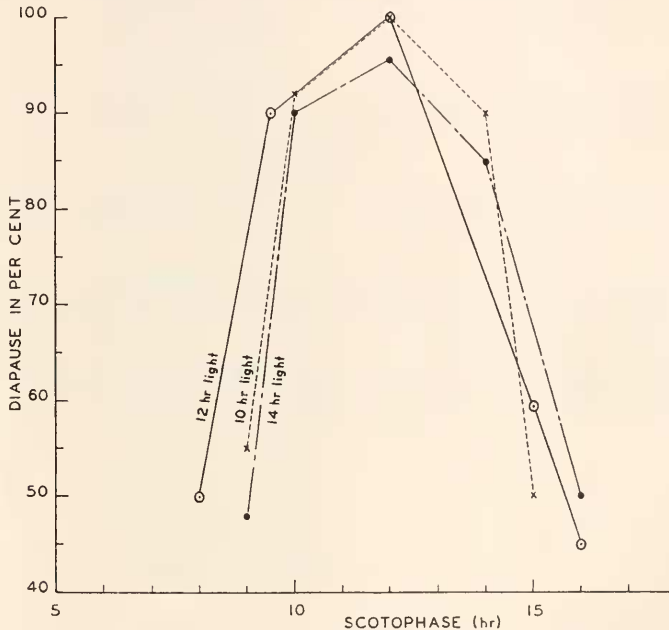


FIGURE 3. The effect of scotophase duration on the incidence of diapause in European corn borer larvae reared under photophases of 10, 12, or 14 hours.

per cent. The three response curves shown in Figure 3 did not prove to be different to a statistically significant degree. Maximum diapause incidence was induced by a 12-hour scotophase in each case.

Diapause incidence among borers reared under scotophases of 10, 12, and 14 hours, combined with a wide range of photophases, was also measured (Fig. 4). A 12-hour scotophase was significantly more effective than 10- or 14-hour scotophases. The 12-hour scotophase induced a high incidence of diapause when it was combined with any photophase of from 5 to 18 hours duration. A photophase of more than 28 hours was required to reduce the diapause incidence to below 50%, when the experimental photoperiod included a scotophase of 12 hours.

Phase durations required to induce a diapause incidence of 90 or more per cent are shown in Figure 5. The 90% response curve describes an ellipse, within which

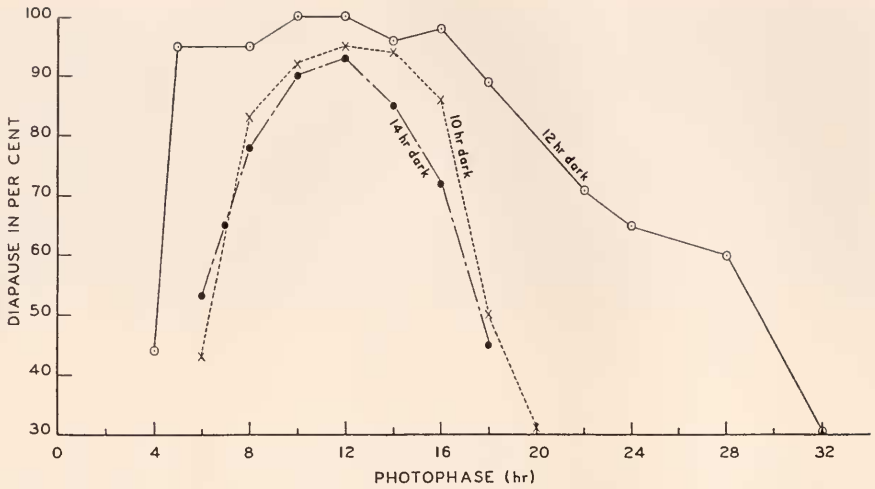


FIGURE 4. Effect of photophase duration on the incidence of diapause among European corn borer larvae reared under scotophases of 10, 12, or 14 hours.

diapause incidence was greater than 90%. The long axis of the ellipse lies along the 12-hour scotophase coordinate, and the widest part of the response zone falls between the 11- to 13-hour photophase and 10- to 14-hour scotophase coordinates. These results, combined with those shown in Figures 3 and 4, suggest that diapause is most efficiently induced by a 24-hour photoperiod containing a 12-hour scotophase.

The characteristics of photoperiods inducing a high incidence of diapause in the

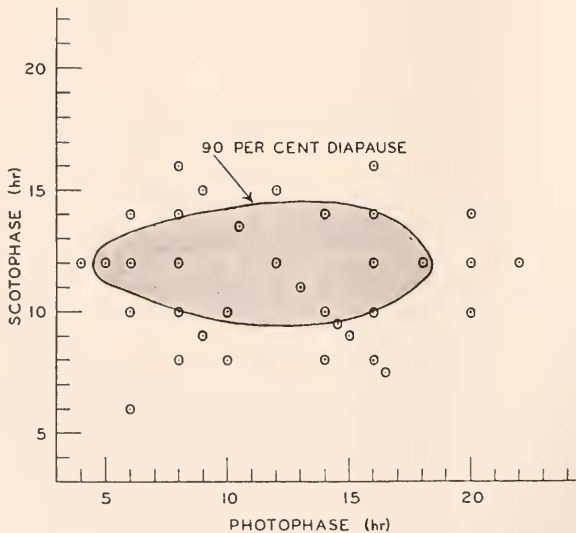


FIGURE 5. Photoperiodic requirements for the induction of a 90 or more per cent incidence of diapause among European corn borer larvae. (Plotted points represent photoperiods tested experimentally.)



European corn borer cover a much wider range of phases than has been found in some other lepidopterous species (Dickson, 1949; Bull and Adkisson, 1960). The response range of the mite, *Metatetranychus ulmi*, is fundamentally different from those of the lepidopterous species tested, in that long scotophases (12 to 24 hours) tended to promote diapause incidence, and long photophases tended to suppress diapause. A 12-hour scotophase was found to be as effective as a 24-hour scotophase, indicating a very broad range of maximum effectiveness (Lees, 1953, 1955).

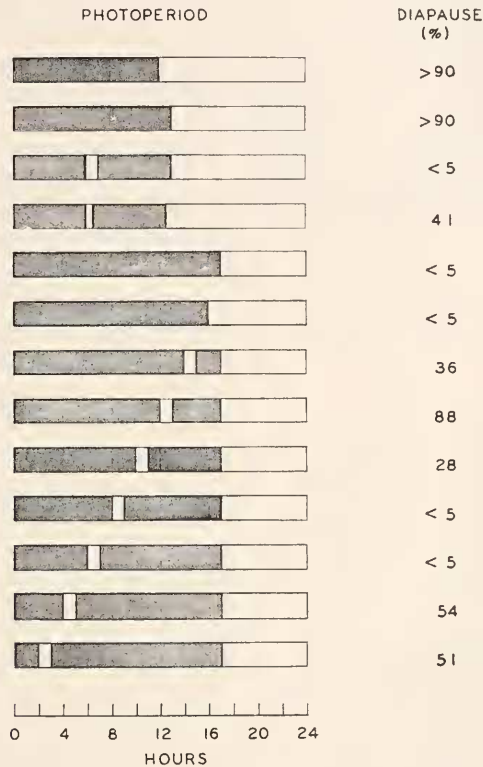


FIGURE 6. Effect of one-hour light interruptions of the scotophase on diapause induction in the European corn borer (24-hour photoperiod).

Photoperiodic induction of diapause in the European corn borer is dependent upon the actual duration of the photoperiodic phases, with the duration of the scotophase being far more critical than that of the photophase. This situation appears to prevail in other photoperiodically responsive arthropods as well. Lees (1953) found that a scotophase of 12 hours would induce the production of diapause-eggs in *Metatetranychus ulmi* even when accompanied by photophases up to 36 hours long. Tanaka (1950), working with the Chinese Tussar-silkworm *Antheraea pernyi*, reported that a 13-hour scotophase was effective in inducing diapause under photoperiods containing photophases as long as 59 hours. All of these results indicate that, although minor species differences exist, diapause in

"long-day" insects is a response to scotophases of about 12 hours duration, within a surprisingly broad range of photophases.

A series of experiments was run to test the effect of interrupted scotophases on the incidence of diapause (Fig. 6). In the first group of such experiments, an hour of light was inserted in the middle of the 13-hour scotophase of a 24-hour photoperiod. Diapause incidence was negligible under such a photoperiodic regime, whereas an uninterrupted scotophase of 12 or 13 hours induced a diapause incidence of well over 90%. Interruption of the scotophase by as little as 0.5 hour of light greatly reduced diapause incidence. The sensitivity of the borer to an interruption of the scotophase is greater than that reported for a number of other insect species, in which interruptions of from two to three hours were required to reduce the diapause-inducing effect of a 12-hour scotophase (Dickson, 1949; Lees, 1953; Danilyevsky and Glinyanaya, 1950; Bunning, 1960).

Scotophases of 17 hours in a 24-hour photoperiod were systematically interrupted by a one-hour light period (Fig. 6). Uninterrupted scotophases of 16 and 17 hours did not induce a significant level of diapause. When the one-hour light break came at the end of 10, 12, or 14 hours of dark, a significant incidence of

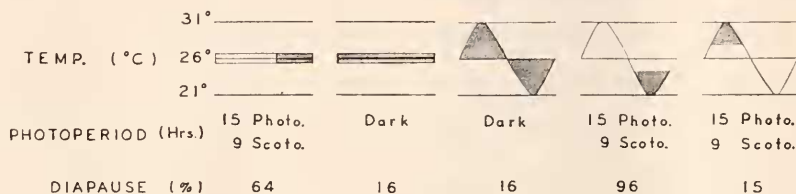


FIGURE 7. Effect of combined photoperiod and thermoperiod on the incidence of diapause in the European corn borer.

diapause was induced. Once again, the highest incidence was associated with 12 hours of uninterrupted dark. When the interrupting light occurred after either 6 or 8 hours of dark, no diapause was observed. After either two or four hours of dark, an hour of light followed by 14 or 12 hours of dark resulted in a diapause incidence of about 50%. Apparently, a 12-hour dark period is less effective if preceded by a four-hour dark period than if followed by a four-hour dark period.

Using the cabbage worm, *Pieris brassicae*, Bunning and Joerrens (1960) also determined the effects of interrupted scotophases on diapause incidence. Although the experimental insect was also a "long-day" form, systematic interruption of the scotophase with one-hour light periods produced a different pattern of response. They found that diapause was prevented by light interruptions occurring about 15 hours after the beginning of the photophase, regardless of the total length of the scotophase or the position of the interruption within the scotophase. The results with the European corn borer (Fig. 6) show no such relationship, and diapause incidence is obviously dependent upon an uninterrupted dark period of about 12 hours, with the response being only slightly influenced by the position of the 12-hour dark period within the total photoperiod.

The previous finding that diapause incidence under a given photoperiod is temperature-sensitive (Beck and Hanec, 1960), and the finding that diapause incidence is closely dependent upon the duration of the scotophase, led to the



hypothesis that the temperature sensitivity of the diapause reaction is associated with the temperature during the scotophase. This was tested by the experimental series shown in Figure 7. Low temperatures during the 9-hour scotophase caused a very high incidence of diapause, whereas low temperatures during the photophase produced no more diapause than did the control conditions of continuous dark. The results clearly imply that diapause induction in the European corn borer involves a scotophasic temperature sensitivity.

Because of the reported effects of light and photoperiodicity on growth rates and a variety of morphogenic processes (Muller, 1957, 1958, 1960; Ball, 1958) growth records were maintained in all experiments conducted in this study. In an earlier paper (Beck and Hanec, 1960) it was reported that the series of 24-hour photoperiods tested had no measurable effects on the rate of larval growth of the European corn borer. In the present study, a much wider range of photoperiods was studied, but no growth rate effects were detected. At the rearing temperature employed (30° C.) the larvae attained the fifth instar at an age of about 10 days, prepupal stage at 11 to 13 days; pupation occurred from the thirteenth day, and pupation of the non-diapause portion of the experimental populations was 50% completed by about the seventeenth or eighteenth day. This developmental schedule did not appear to be materially altered by any photoperiod tested, except, of course, that the larvae entering diapause did not pupate.

#### DISCUSSION

From the results of this study, some of the characteristics of the photoperiodic reactions of the European corn borer may be deduced. The induction of diapause is obviously associated with the periodic occurrence of 12-hour scotophases during the 11-day developmental period of the larva (at 30° C.). Within rather broad limits, the actual duration of the scotophase was found to determine the incidence of diapause, regardless of the relative proportion of the photoperiod occupied by the scotophase.

Following exposure to a 12-hour scotophase, the borer is apparently refractory to a second dark "stimulus" for a period of from 4 to 5 hours. This interpretation is based on the results of two types of experiments. First, the shortest diapause-inducing photophase was found to be between 4 and 5 hours in duration (Fig. 4). This might be interpreted as meaning that the "light reaction" requires from 4 to 5 hours for effective activation, were it not that the results of the interrupted scotophase experiments are inconsistent with such a conclusion (Fig. 6). These experiments showed that light durations of an hour or less are effective. They also indicate the existence of a dark-refractory period following a 12-hour scotophase; the photoperiodic regime of 12 hours dark, 1 hour light, 4 hours dark, and 7 hours light resulted in a diapause incidence of 88%. Statistical analyses showed that such a response was not significantly different from the response to a simple photoperiod composed of a 12-hour scotophase and 8-hour photophase. It would seem that the borers failed to respond to the added four hours of dark.

The effect of a photophase of several hours is apparently no more than the effect of a photophase of but one hour. Diapause incidences of about 50% were obtained when a 17-hour scotophase was divided into a short period (two- or four-hour), an hour of light, and a long dark period (14- or 12-hour) (Fig. 6). Since

a significant incidence of diapause was observed, the short dark period was not followed by a dark-refractory period. The long dark periods had a diapause-inducing effect, as expected, but were followed by a second effective period of light. The over-all effect of such a photoperiodic regime was that there were two effective periods of light (1 and 7 hours) for every effective scotophase, thus reducing the diapause-inducing effect to the photoperiod.

Even with 12-hour scotophases, diapause incidence declined slowly as the length of the total photoperiod exceeded 30 hours (Fig. 4). This effect is probably determined by the number of photoperiods occurring within the 11-day larval developmental period. A 12-hour scotophase combined with a 5-hour photophase produces a 17-hour photoperiod, and 15.5 such photoperiods can occur in 11 days (264 hours). However, when a 12-hour scotophase is combined with a 32-hour photophase, the photoperiod is 44 hours, and only 6.0 such photoperiods can occur in 11 days. The gradual decline of diapause incidence observed with photoperiods of increasing length is interpreted as being explicable on the basis that the incidence of diapause tends to be proportional to the number of 12-hour scotophases experienced during larval development.

Assuming that the induction of diapause is dependent upon the occurrence of a required number of effective scotophases and photophases, it should be possible to devise a system of predicting the incidence of diapause under any given photoperiodic regime. Such a predictive scheme would be based on the accumulation of diapause-effective hours of light and dark over the period of larval development, in a manner comparable to the temperature accumulations that have widespread use in phenological predictions. A number of systems of photoperiodic accumulation were devised. Highly significant coefficients of correlation between per cent diapause and photoperiodic accumulations were obtained by nearly all of the methods tested. However, the schemes were devised to fit the data at hand, and in spite of such bias, serious discrepancies were apparent between "predicted" and observed results in a few crucial experiments. For these reasons, the accumulation methods tried were concluded to be without real meaning. Until more is known about the dynamics of both the "light" and the "dark" reactions, an empirical system of predicting diapause incidence is not likely to be of much fundamental significance.

The over-riding importance of a scotophase of about 12 hours and an effective light flash of only one hour in the induction of diapause may be interpreted to support the conclusion of Lees (1960) that the physiological mechanisms involved behave as an interval timer. The diapause-inducing photoperiods tested in this study were not confined to a periodicity of 24 hours, or any multiple thereof, or to any other specific cycle duration. These findings lend little support to the hypothesis that diapause is in response to the effects of photoperiods on an endogenous circadian rhythm. On the other hand, Figures 3, 4, and 5, above, may be interpreted as lending at least feeble support to the hypothesis that photoperiodic induction of diapause involves a circadian function, in that the most effective range of photoperiods centered around a 12-hour photophase as well as a 12-hour scotophase. The tendency for diapause induction to be associated with a 24-hour periodicity was much less well defined than was its dependence on a 10- to 14-hour scotophase.

Diapause, itself, cannot be a rhythmic function: it occurs but once per individual. Whether or not one or more of the contributing physiological events involves a circadian rhythm has not been demonstrated. Feeding behavior activity cycles have been implicated in the induction of diapause in the beetle, *Leptinotarsa decemlineata* (DeWilde *et al.*, 1959), but the photoperiodic effects could not be fully explained solely on the basis of the effects of photoperiod on feeding. Modification of photoperiodic effects by experimental alteration of feeding behavior was also reported by Muller (1957), who worked with a number of species of the homopterous genus, *Euscelis*. Circadian rhythmicity and an influence of photoperiod on such activities as locomotion and eclosion have also been reported (Harker, 1960a; Pittendrigh and Bruce, 1959). A relationship between circadian cycles, neurosecretory cycles, and diapause induction has thus far eluded demonstration, however. Whether diapause is the result of photoperiodic induction of elaboration of a "diapause hormone" (Hasegawa, 1957; Lees, 1959b; DeWilde and Boer, 1961), or a photoperiodically induced biochemical failure in the morphogenic chain of events remains to be determined.

#### SUMMARY

1. The European corn borer, *Ostrinia nubilalis*, is a so-called long-day insect, larval diapause being induced by naturally occurring photoperiods containing scotophases of from 10 to 14 hours.

2. Diapause induction was found to be dependent upon the actual number of hours of the photoperiodic phases. The duration of the scotophase was far more critical than that of the photophase. A 12-hour scotophase was of maximum effectiveness when combined with photophases of from 5 to 18 hours. Significant incidence of diapause occurred when a 12-hour scotophase was combined with photophases of from 4.5 to 32 hours.

3. Diapause induction is a temperature-sensitive phenomenon, with the incidence of diapause tending to be inversely proportional to the ambient temperatures occurring during the scotophase.

4. Interruption of the scotophase by a one-hour period of light modified the photoperiodic response, the effect depending on the position of the light interruption within the scotophase. The effects were interpreted as a demonstration that the insect's photophasic requirement is satisfied by a one-hour light period, but that longer photophases are normally required because of a dark-refractory period following 12-hour scotophases.

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